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Conference on Surfaces of Biomaterials
Biotechnology--Biointeractions '87

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Presentations given at this conference, held in July 1987 in Cambridge, UK, are discussed. The conference was focused on the ways in which biotechnological products and man-made materials interact physically and chemically with biological environments. Topics of the program were: cell/polymer interactions, blood/surface interactions, innovations--monoclonal antibodies and biosensors, biocompatibility, bioadhesion, and drug delivery systems.

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CONFERENCE ON SURFACES OF BIOMATERIALS BIOTECHNOLOGY--BIOINTERACTIONS '87

1 INTRODUCTION

Biointeractions '87: Surfaces of Biomaterials/Biotechnology, the second of these international conferences, was held at Churchill College, Cambridge, UK, from 6 through 8 July, 1987. The conference was sponsored by the journal *Biomaterials* (Butterworth & Co. Publishers Ltd, UK) and organized by M. Korndorfer of Butterworth with the aid of an international scientific committee. The Office of Naval Research also provided some support for this well-organized and timely conference.

The total attendence of 145 comprised scientists from nine West European countries as well as the UK, Israel, US, Australia, Kenya (Africa), and Bulgaria. About 65 percent of the participants represented academic institutions with the balance from industrial organizations.

The aim of the conference was to enlarge and improve understanding between materials and life scientists by focusing on the ways in which biotechnological products and man-made materials interact physically and chemically with biological environments. Behavior at surfaces strongly influences the effectiveness of implanted materials and such phenomena can be appropriately adapted to specific situations.

The scientific program was arranged in six sessions over 3 days and comprised lectures by keynote speakers, short contributed papers, and poster presentations. The keynote papers will be published in a special issue of *Biomaterials* in about 6 months.

The program format consisted of the following topics:

- Cell/polymer interactions;
- Blood/surface interactions;
- Innovations: Monoclonal antibodies and biosensors;
- Biocompatability;
- Blondhesion;
- Drug delivery systems.

Keywords: Artificial tissue, Tissue grafts, Surgical Implantation, (GIC/AW)

Following are discussions of papers presented under each of the topics.

2 CELL/POLYMER INTERACTIONS

In vivo Cell/Polymer Interaction

Studies of *in vivo* cell/polymer interactions were presented by J.M. Anderson (Institute of Pathology, Case Western Reserve University, Cleveland, Ohio). Anderson stated that the traditional pathway for wound healing (injury, acute inflammation, chronic inflammation, tissue granulation and fibrosis) also occurs with implants in the body. This course of events is also called a foreign body reaction. Fibrosis occurs if the implant is not biodegradable. If the implant is biodegradable, an acute response occurs with swelling and cellular infiltration. A consistent finding in the evaluation of retrieved human implants by Anderson and his group was the presence of macrophages and foreign body giant cells at or near the surface of the prostheses or devices. In order to investigate this phenomenon, Anderson's group has studied cellular adhesion of several polymers in their cage implant system in which they assessed the biocompatability of *in vivo* implants by examining the exudate that surrounds the implant. The group monitored polymorphonuclear (PMN) alkaline phosphatase as well as mononuclear and cathepsin acid phosphatase of the exudates. Their studies showed distinct differences in cellular adhesion between the various polymers. Also, surface concentrations of cells, measured as cells per square mm, were different for the respective polymers. It was found that macrophages, when activated by the polymers, can secrete about 100 components such as polypeptide hormones, and growth factors (interleukins, platelet-derived growth factor, etc.). Anderson's group is engaged in extensive studies of selected aspects of macrophage activation, following adhesion and the role of cell-material, cell-protein, and cell-cell interactions as they relate to the biocompatability of polymers.

The deposition of endothelial fibronectin onto polymeric surfaces was

reported by P.B. van Wachem (Department of Chemical Technology, Biomaterials Section, Twente University of Technology, Enschede, the Netherlands). Van Wachem mentioned that the clinical results with small-diameter vascular grafts made from polymers such as Dacron (PETP, polyethylene terephthalate) and Teflon are generally disappointing, mainly because of occlusion due to thrombus formation. He also stated that since vascular endothelium represents a unique nonthrombogenic surface, he considered that endothelial cells are the first logical choice for lining small-diameter vascular grafts. Therefore, van Wachem and his group carried out a systematic *in vitro* study of the interaction of human endothelial cells (HEC) with polymers with the aim of developing grafts which could promote overgrowth of endothelial cells.

These investigators had previously found that HEC, suspended in a culture medium containing human serum, adhered to moderately wettable polymers such as tissue culture polystyrene (TCPS). Reduced adhesion or no adhesion was observed upon the more hydrophobic polymer PETP and the very hydrophobic polymer, fluoroethylene-propylene copolymer (FEP, a Teflonlike polymer). Recently, van Wachem and his group also observed that TCPS adsorbs more of the adhesive protein fibronectin from the culture than PETP or FEP, although this amount was small compared to, for example, adsorbed albumin. They considered it unlikely that this relatively small amount of fibronectin was responsible for the adhesion, spreading, and proliferation of HEC. Therefore, they studied the deposition of endothelial cell-derived fibronectin to TCPS.

The use of culture medium containing fibronectin-depleted serum and the application of an enzyme immunoassay in which a monoclonal antifibronectin antibody was used, enabled van Wachem and his coworkers to show that cellular fibronectin is deposited upon TCPS during adhesion and spreading of HEC. After seeding of HEC onto this polymer, increasing amounts of fibronectin are deposited both with increasing cell density and increasing incubation time. Their results indicate that the ability of depositing cellular fibronectin onto a polymeric surface is a condition for the spreading and proliferation of HEC.

Bacterial Adhesion to a Polystyrene Surface

Studies of bacterial adhesion to a modified polystyrene surface were reported by M.A. Khan (Department of Pharmacy, University of Nottingham, UK) in collaboration with A. Brown (Department of Chemistry, University of Manchester, UK). Bacterial adhesion is considered to be an important step in the pathogenesis of infections associated with prosthetic implants and medical devices. These infections, according to Khan, are predominantly associated with coagulase-negative *Staphylococci*, notably *Staphylococcus epidermis*. *In vitro* studies have indicated that both the hydrophobic interactions and surface charge properties of the bacteria and the surface chemistry of the biomaterial markedly influence bacterial adherence. In this report, Khan and coworkers investigated the adhesion of a wide range of highly characterized clinical isolates of *Staphylococcus epidermis* to oxygen-glow-discharge-treated polystyrene surfaces characterized by means of modern surface analysis techniques.

The surface chemical modification of the polystyrene films was monitored by x-ray photoelectron spectroscopy (XPS) and static secondary ion mass spectrometry (SSIMS). The high sensitivity and degree of molecular specificity of SSIMS provides, according to Khan, a novel perspective on the dramatic changes in the surface chemical structure, both at the elemental and molecular levels, as induced by plasma treatment. The bacterial adhesion was elucidated by the determination of the extracted ATP of the adherent bacteria and was found to be highly dependent on the strain of *S. epidermis* and the surface hydrophobicity of the bacteria as derived by hydrophobic interaction chromatography. In addition, the degree of oxidation of the polymer surface also illicited a profound modification

of the bacterial adhesion in certain strains. Khan thinks that these cellular adhesion phenomena may be interpreted in terms of the surface chemical phenomena of both the bacterial interface and with the application of the SSIMS technique, of the interfacial chemical character of the modified polymer surface.

Effect of Implantation Site on Cell/Polymer Interactions

Studies on the effect of implantation site on mononuclear and multinucleated cell/polymer interactions was presented by D. Bakker (Ear Nose and Throat Department, University Hospital and the Laboratory for Electron Microscopy, State University, Leiden, the Netherlands). Bakker stated that the rate of hydroxyapatite biodegradation appears to vary with implantation technique. In order to evaluate the effect of implantation site on phagocyte/polymer interactions, Bakker and coworkers implanted three alloplastic tympanic membrane materials: (1) polypropylene oxide; (2) Estane 5714, F1 polyether urethane; and (3) a polyether polyester copolymer. A total of 840 porous specimens were implanted in rat middle ears: in the tympanic membrane, submucosally, and between middle ear bone and adjacent muscle tissue. Survival times ranged from 1 to 52 weeks, and light microscopy and autoradiography, transmission and scanning electron microscopy, and x-ray microanalysis served as evaluation techniques. It was found that all implantations resulted in the presence of mononuclear and multinucleated phagocytes. Morphometry showed that for all three biomaterials the total volume of phagocytes present at the site implantation decreased in the following order: (1) adjacent to muscle tissue; (2) submucosally, and (3) in the tympanic membrane. Biofragmentation or biodegradation, which may have resulted from polymer/phagocyte interactions (according to Bakker), occurred for all tested biomaterials, but were qualitatively and quantitatively most prominent when the polymers were in contact with muscle tissue. Transmission electron microscopy showed, at all implantation sites, intracellular material derived from the three polymers. Findings with respect to polypropylene oxide also suggested extracellular degradation.

This study showed that phagocyte activity was greatest when implants contacted muscle tissue, and confirmed previous reports by Bakker and others of stimulated phagocyte activity by cellular irritation. Bakker thinks that the observation that the implantation site affects phagocyte activity stresses the importance of studying the biological performance of an implant material at the site of the clinical implantation.

Protein Adsorption at the Interface

Protein adsorption at the interface between charged polymer substrata and migrating osteoblasts is being studied by J.E. Davies (Department of Anatomy, University of Birmingham Medical School, UK). Davies and his group have recently shown that both the migratory morphology and synthetic activity of osteoblasts (OB's) are influenced by the substrata they colonize *in vitro* and that this differential behavior is related to the surface charge of the substratum. Their experimental system is based on the migration of primary rat calvarial osteoblasts onto polystyrene ion exchange resin beads of either positive or negative surface charge. Using this system, Davies and coworkers have speculated that the behavior of OB's may be influenced through the intermediary of charged double layers or adsorbed serum proteins originating in the culture medium. In fact, the substrate surface is exposed to the proteins from the fetal calf serum (FCS) in the medium for a significant time before the OB's migrate over it, allowing the adsorption of proteins and other macromolecules. In the present study, Davies reported on the difference in proteins adsorbed to these polymer substrata of either positive or negative surface charge and linked this to OB behavior.

The methods used by Davies and his group in the present study were: (1) elution of adsorbed protein from substrata using sodium dodecyl sulfate (SDS) and analysis using polyacrylamide gel

electrophoresis (PAGE), and (2) immunolabeling FCS proteins (horseradish peroxidase), with subsequent observation by Light microscopy (LM) or transmission election microscopy (TEM). PAGE analysis revealed reproducible differences in the protein population eluted from the charged substrata. While these protein/peptide bands were constant for each substrate, they differed from those seen in FCS. As the majority of proteins carry a net negative charge at physiological pH, Davies thinks that it would seem likely that there would be preferential or increased adsorption of serum proteins on positively charged substrates. However, the preliminary results do not indicate such a clear cut situation, since different protein/peptide populations are desorbed from different negatively and positively charged substrates. There does not, therefore, appear to be a correlation between the differences in the bands seen and the charge of the substrate, indicating that other factors such as surface exchange ions may influence protein adsorption. On both strongly and weakly negatively charged beads these investigators found that there was an increased number of bands seen in the molecular range lower than albumin when compared with the bands seen from native FCS, and this number of bands was decreased with eluants from positively charged beads. Davies found that there appeared to be a correlation between the number of bands seen in the high molecular weight range and the ease with which OB's cover their surface. Davies and his group are currently involved in identifying the different bands seen on the gels. Immunolabeling has demonstrated a definite layer of adsorbed protein on the surface of the substrates, and Davies is currently addressing the problem of labeling protein beneath the OB population.

3 BLOOD/SURFACE INTERACTIONS

Albumin Adsorption on Alkyl Derivatized Polyurethanes

A report on albumin adsorption on alkyl polyurethanes was presented by W. Pitt, working under the direction of T.G. Grassel and S.L. Cooper (Department of Chemical Engineering, University of Wisconsin, Madison). The attachment of alkyl chains to polyurethanes has been observed to increase the affinity of serum albumin to alkyl polymer derivatives with alkyl groups on the polymer surfaces. However, the molecular mechanisms producing this increased surface affinity have not been fully investigated. Therefore, in the present study, Pitts and coworkers prepared polyurethanes based on a 3/2/1 mole ratio of methylene bis (p-phenyldiisocyanate) (MDI), butanediol, and 1000-molecular-weight polytetramethyleneoxide in which 10 percent of the urethane nitrogens were grafted with linear alkyl chains of carbon lengths 18, 12, and 2. Two monomeric fractions of human serum albumin were adsorbed to these polymers in a laminar flow cell. One albumin fraction had no free fatty acid bound in the alkyl fatty acid binding sites of the protein, while the other had the alkyl binding sites blocked with 6.5 moles of stearic acid per mole of albumin. The kinetics of protein adsorption was recorded continuously using Fourier transform infrared spectroscopy and internal reflection optics. Analysis of the initial adsorption rates indicated that increased albumin affinity to the C-18 polymer derivative was due to both nonspecific binding to the more hydrophobic surface and specific binding at the alkyl binding sites. In addition to blocking the alkyl binding sites, addition of stearic acid to albumin makes the protein less susceptible to denaturation. When albumin was adsorbed to the underivatized polyurethane, the more stable albumin containing stearic acid adsorbed more slowly than the less conformationally stable albumin without stearic acid. This suggests, according to Pitt, that proteins which are less stable with respect to denaturation may adsorb more readily to biomaterial surfaces.

Evaluation of Polyester Arterial Grafts Coated with Albumin

A study of the evaluation of polyester arterial grafts coated with albumin was reported by R. Guidon (St. Francis of

Assise Hospital and Laval University, Quebec City, Canada). Previous *in vitro* studies indicated that the type of chemical used to cross-link albumin-coated polyester arterial prostheses may influence the rate of bioerosion of the albumin layer *in vivo*. In the present study, four series of implantations in the thoracic aorta of dogs for scheduled periods of 4 hours to 6 months were conducted using 1.6- and 2.5-percent glutaraldehyde and 0.2-M carbodiimide as the alternative cross-linking agents plus a nonalbuminated preclotted polyester prosthesis which served as control.

The pathology of the explanted grafts revealed that in the short and medium term, the rate of healing and the extent of tissue ingrowth was dependent, initially, on the presence of--and later, on--the rate of bioerosion of the albumin layer. After three months *in situ*, the prostheses coated with albumin cross-linked with 1.6 percent glutaraldehye and carbodiimide had healed more rapidly and were invaded by more extensive tissue ingrowth than the one cross-linked with 2.5-percent glutaraldehyde or the preclotted control. Also, the migration of cells over the carbodiimide-treated surface was the most fully developed and most regularly organized of all four series. Immunostaining revealed that the presence of glutaraldehyde induced an inflammatory response which failed to support the growth of normal luminal cells with the endothelial phenotype.

Research on Functional Polymers and Their Biomedical or Biotechnological Applications

Studies by M. Josefowicz (Laboratoire de Recherches sur les Macromolecules, C.N.R.S. UA 502, Université Paris-Nord, France) have shown that functional polymers, i.e.--synthetic or artificial polymers--substituted with specific chemical groups carried by the macromolecular chain, interact specifically with living systems. Josefowicz and his group have carried out extensive research on functional polymers and their biomedical or biotechnological applications.

Polymers containing aryl sulphonate and carboxyl groups have been shown by these investigators to interact specifically with antithrombin III and certain serine-proteases in the coagulation of blood. These biomaterials are either cross-linked polymers (polystyrene-polysaccharide-polyethylene, etc.) or soluble biodegradable polysaccharides. Thus, these biomaterials in contact with blood are nonthrombogenic because they are able to catalyze the inhibition of the coagulation cascade. Some functional polymers have been prepared by this group in order to specifically interact with various components of the immune system--for example, with proteins involved in the complement system and with antibodies developed by hemophiliac A and Systemic Lupus Erythematosus patients. Also, it was found that such functional polymers may interact with growth factors and cells. Therefore, according to Josefowicz, these biomaterials are candidates for use in biotechnology.

An Isotopic Method to Estimate Density and Distribution of Heparinlike Materials

Heparinlike materials, characterized by a defined superficial density of functional groups which activate antithrombin III (AT III), have been found to inhibit thrombin as soon as it appears when they are in contact with blood. An isotopic method has been developed by J. Caix (Laboratoires de Biophysique et d'Immunologie Cellulaire, Université de Bordeaux II, France) to estimate the density and to visualize the distribution of the affinity sites concerned, directly with AT III labeled with ^{125}iodine, and indirectly with an anti-AT III monoclonal antibody [illegible] with Indium III. The work reported by Caix was a collaborative project with scientists at the Molecular Biology Laboratory, University of Paris-Nord, France.

Caix and her coworkers found that AT III is able to adsorb specifically on modified polyethylene tubes. However, when the polystyrene coating of their inner surface is only sulphonated, the AT III is no longer recognized by its antibody. Caix thinks that these results

probably indicate differences between adsorption mechanisms on both types of modified polyethylene (Type A and Type B).

Improving Biocompatibility of Vascular Graft Materials

Improvement of biocompatibility of vascular graft materials by *in vivo* lining with autologous endothelial cells was reported by M. Kadletz (Department of Surgery, University of Vienna, Austria). Since the introduction of endothelial cell seeding in 1978, numerous studies have demonstrated the long-term benefit of this technique in terms of the reduction of the thrombogenicity of artificial grafts. However, no improvement of short-term patency can be achieved by single-staged seeding due to the low inoculum, according to Kadletz. Therefore, she and her coworkers focused on an alternative method in the form of immediate endothelial cell lining by means of cell culture. For this purpose, these investigators established human saphenous vein endothelial cell (EC) cultures. Cells were seeded onto artifical grafts. The addition of EC growth factor was found to increase the number of EC/cm^2 grafts. Subsequently, the grafts were perfused in a mock circulation unit, imitating the shear forces of the superficial femoral artery. After 24 hours of pulsatile prefusion, 53 percent of EC were still adherent, while after 48 hours only 36 percent of the preperfusion cell number could be found. However, in spite of this appreciable cell loss, most of the grafts displayed closed EC monolayers, as seen by scanning electron microscopy. Kadletz thinks that the results of these studies indicate that *in vitro* lining offers a promising technique to improve the thrombogenic surface of artificial vascular grafts and that it could soon develop into clinical routine wherever a cell laboratory is available.

Blood Compatibility of Cross-Linked Polyether Blends

A report on blood compatibility of cross-linked polyether blends was presented by J.G.F. Bots (Department of Chemical Technology, Biomaterials Section, Twente University of Technology, Enschede, the Netherlands). Cross-linked blends of polyethylene oxide (PEO) and polypropylene oxide (PPOX) have been studied by Bots and his group for application as biomaterials; for example, as small-diameter blood vessel substitutes. In previous work on the relationship between platelet adhesion and type and amount of polyether segments in copolyether urethanes it had been observed that an increase of the polymer content resulted in a reduced platelet adhesion. Because PEO had been found to be the most active in suppressing platelet adhesion compared to PPOX and polytetramethylene oxide (PTMO), Bots and his group considered that a combination of PEO with PPOX or PTMO might lead to materials with a good blood compatability. PEO could be the blood compatable, hydrophilic component and PPOX or PTMO the more hydrophobic and mechanically stronger one. His report is therefore concerned with studies to assess this hypothesis.

Films of blends of PEO and PPOX were cross-linked by ultraviolet irradiation in the presence of dicumyl peroxide (DCP). Water content (after equilibration) and mechanical properties of the cross-linked films were varied by changing the blend composition or the irradiation time. Surface characterization showed that in the phase-separated cross-linked PPOX/PEO films more PEO was present on the surface than in the bulk. It was also found that on equilibration with water, PEO became hydrated, thereby losing its crystalline character.

Interaction of the PPOX/PEO cross-linked blends with blood was studied by measuring kallikrein generation, APTT values, and platelet adhesion. Among the blends studied, the PPOX/PEO at 90/10 and 80/20 were the most blood compatible. They showed a low kallikrein generation and platelet adhesion and a high APTT value.

According to Bots, the results with the polyether blends obtained so far indicate the importance of an increased PEO surface concentration in combination with a transformation of the PEO phase into the hydrated form, thus creating a

hydrophilic, noncrystalline and blood-compatible surface.

Adsorption of Thrombin to Modified Polystyrene Resins

Studies of the adsorption of thrombin to modified polystyrene resins and competition with other plasma proteins was presented by C. Boisson (Centre Scientifique et Polytechnique, Université Paris-Nord, Villetaneuse, France). Boisson works in the group of J. Josefowicz. This was a collaborative project with J.L. Brash (McMaster University, Hamilton, Canada). These investigators had previously found that polystyrene resins containing sulphonate and sulphonate-arginylmethyl ester sulphamide groups simulate the binding site of antithrombin III (AT III) for thrombin so that such resins should have a high affinity for thrombin. In the present study, work was undertaken to obtain detailed quantitative information on the binding of thrombin to these resins, particularly in a plasma medium so that the selectivity of the resins for thrombin in the presence of other proteins could be assessed.

In this study three resins were used: a 95 percent sulphonated polystyrene and two arginyl derivatives of sulphonated polystyrenes, one having a low (58 percent) and the other a high (78 percent) degree of substitution of arginyl groups. The binding of thrombin was studied in two media: (91) isotonic tris buffer, pH 7.4, and (2) a modified plasma from which the fibrinogen (Fg) was removed by the snake venom, Arvin, and the AT III (which otherwise would be complexed to the thrombin) was removed by treatment on a Sepharose-heparin affinity column. To determine the quantity of thrombin bound to the resins, thrombin was labeled with ^{125}I, mixed with either the isotonic buffer or the plasma, and incubated with the resins at 4° C. The specific surface areas of the resins were estimated from measurements of albumin adsorption under conditions leading to close-packed monolayer formation.

The adsorption isotherms of thrombin both in buffer and in AT III/Fg-free plasma were found to be Langmuir-like. On this basis, apparent affinity constants were estimated. The quantities of thrombin adsorbed at the isotherm plateau were in the monolayer range. Also, the values in plasma were of the order of 70 percent of those in buffer. According to Boisson, these data confirm the high affinity of these materials for thrombin even in a complex medium containing numerous other potentially competing species--a finding important for eventual practical application.

4 INNOVATIONS: MONOCLONAL ANTIBODIES AND BIOSENSORS

Biocatalysis in Nonaqueous Solvents

A review of biocatalysis in nonaqueous solvents was presented by A. Klibanov (Department of Applied Biological Sciences, Massachusetts Institute of Technology, Cambridge). Klibanov is well known for his work in this field, which may well be one of the most exciting and promising emerging trends in biotechnology. Enzymatic production of materials is usually carried out in aqueous solvents because organic solvents, in general, lead to denaturation of enzymes which are proteins. However, Klibanov has found in his studies that water (aqueous solvents) may not be the best medium for the catalytic function of enzymes. Many organic synthesis reactions are insoluble in water, stability appears to be greater in organic solvents, and there is no problem of bacterial contamination which occurs in aqueous solvents. Klibanov pointed out that it is not a question of whether water is required for enzymatic activity but rather how much water is necessary. He stated that only a monolayer of water is all that is necessary and that the rest of the milieu surrounding the enzyme can be organic. Another important point is the nature of the organic solvent. If it is very hydrophobic, the required monolayer surrounding the enzyme would be removed and the enzyme inactivated. Therefore, the nature of the organic solvent used in the reaction is very important. An interesting point mentioned by Klibanov is that enzymes have pH memory. Therefore, the pH of the reaction medium

should be about the same as the pH of the aqueous medium from which the enzyme was isolated in order to maintain enzymatic activity in a nonaqueous medium. He also found that the obligatory amount of water surrounding the enzyme in organic solvent may be even less than a monolayer, and this can be at a level of 300 moles of water/mole of enzyme. In addition, stability of enzymes in organic solvents--in particular, thermal stability--can be quite marked. Furthermore, it appears that enzymes in organic solvents can catalyze new reactions. For example, lipase catalyzes only one reaction in aqueous medium but in organic medium is capable of catalyzing several reactions, according to Klibanov. Lipase in organic solution can be used to produce biosurfactants. Klibanov also found that enzymes retain their catalytic activity in organic solvents because their conformation remains essentially the same as in aqueous solvents. The reason, according to Klibanov, is that an enzyme cannot change its conformation in organic solvent because, without water, it is too rigid to change its conformation in spite of the minimal amount of water surrounding the enzyme in the organic solvent. Thus the biocatalytic production of new materials (optically active synthons and polymers, phenolic resins, selectively modified sugars, biodetergents, etc.) in nonaqueous media are already in progress by Klibanov and his group as well as others and are assuming increasing importance in the area of biotechnology.

An Enzymatic System for Removal of Heparin

An enzymatic system for the removal of heparin was presented by H. Bernstein (Department of Applied Biological Sciences, Massachusetts Institute of Technology, Cambridge). Extracorporeal medical machines (for example, pump oxygenator, artificial kidney) rely on systemic heparinization to prevent thrombus formation in the extracorporeal device. A novel approach that enables heparin to be specifically removed from blood after it has served its purpose in the circuit and before it returns to the patient has been developed by Bernstein and coworkers. A reactor containing immobilized heparinase--a specific heparin-specific enzyme--is placed at the end of the extracorporeal circuit. Heparinase was immobilized to spherical agarose particles using cyanogen bromide activation. The immobilized heparinase was placed in an arterial blood filter which had been fitted with an internal recirculation line to maintain the contents of the reactor well mixed.

A mathematical model was developed to describe the degradation of heparin by the reactor *ex vivo*. Heparin exists in blood in two forms--free and bound to the protein antithrombin. The model accounts for the binding reaction of heparin to antithrombin. The enzyme reactor was modeled as a steady-state continuous-stirred tank reactor. The model has no adjustable parameters and was able to predict the single-pass conversions within 15 percent for three different animals and 12 perfusions, according to Bernstein. At a blood flow of 120 ml/min, 30 to 60 percent of the heparin was degraded after a single pass through the device. The conversion of heparin was determined primarily by the immobilized heparinase concentration, the plasma antithrombin concentration, and the plasma heparin concentration.

Concepts and Technology of Biosensors

A general introduction to the concepts and technology of biosensors including some discussion of the need for sensors, the range of analytes that can be measured, the application areas, the construction of sensors with a biological recognition/response system, and the range of transducer technologies that are potentially amenable to sensor use was presented by C.R. Lowe (The Biotechnology Center, University of Cambridge, UK). Examples of sensor technologies for the monitoring of substrates, proteins, and immunoanalytes were also given. For example, the development of a novel amperometric sensor for glucose and other substrates which incorporates oxidases in conducting organic polymers and of a miniaturized surface conductivity device for

monitoring analytes such as urea was discussed by Lowe. In addition, Lowe spoke about devices capable of detecting proteinaceous analytes based on sophisticated optical techniques including use of an optoelectronic, sensitized semiconductor, conductimetric immunosensor. A review of biosensors containing much of the material presented by Lowe is available in *ESN* 40-9:301-305. In addition, Lowe's presentation will be published in full in a special issue of *Biomaterials*.

Optical and Electrochemical Detection of Immobilized DNA

A report on studies concerned with the optical and electrochemical detection of immobilized DNA was presented by P.J. Warner (Biotechnology Center, Cranfield Institute of Technology, Cranfield, UK). Optical and electrochemical methods of DNA detection were examined by Warner and coworkers using several enzyme labels including horseradish peroxidase, alkaline phosphatase and glucose oxidase. DNA was labeled with these enzymes using a method based on ionic binding of an amine/enzyme complex to the negatively charged DNA. Lambda DNA was used as a model system by immobilization onto nitrocellulose filter paper and hybridization to its labeled complementary strand.

The optical system employed two chemiluminescence reactions using luminal and methylacridone. Light produced by oxidation reactions was detected using a purpose-built inexpensive ($150) luminometer. Peroxidase-labeled DNA was detected by chemiluninescence of luminol in a diethanolamine buffer (100 mM, pH 9.0) in the presence of excess hydrogen peroxide. Detection limits were 1 nanogram of DNA with a response time of approximately 5 minutes.

Glucose-oxidase-labeled DNA produced hydrogen peroxide in the presence of excess glucose. This was detected using the luminometer by the chemiluminescence of methylacridone (1 mM, pH 12.0). According to Warner, initial results indicated that this approach had sufficient sensitivity for single-copy gene detection (1 picogram of DNA), with a response time of 10 minutes.

The electrode approach employed a fluoride-ion-selective electrode. Peroxidase cleaves fluoride ions from organofluoro compounds such as 4-fluorophenol in the presence of hydrogen peroxidase. Peroxidase-labeled DNA was readily detected with a rapid response time (10 to 30 seconds). The detection limit was only 10 nanograms of DNA, but the use of stripping voltammetry to detect chloride ions is presently being investigated by Warner and coworkers, and he thinks that this may greatly increase the sensitivity of the method. The expectation is that these new approaches to DNA detection will be applicable to the production of a rapid and cheap DNA biosensor. However, progress towards a final configuration is likely to be limited by the slow hybridization kinetics associated with DNA, according to Warner.

Analysis of Immunological Reactions by Optical Biosensors

The analysis of immunological reactions on biochemically sensitized surfaces by optical biosensing techniques was discussed by U. Strange (Department of Geology and Physical Sciences, Oxford Polytechnic, Oxford, UK). The research presented was a joint project with N. Groome (Department of Biology, Oxford Polytechnic) and L. Tarassenko (Department of Engineering, Oxford Polytechnic). The intensity of light reflected by a biochemically sensitized surface can be modified when molecules in a chemical solution bind to the surface. According to Strange, the change in reflectance can be measured by an optical sensor and used to quantify immunological reactions on surfaces. Strange and coworkers have developed an optical instrument using laser light which is capable of employing two independent methods to monitor these reflectance changes. The intensity change of the reflected light may be measured by Brewster angle reflectometry or the shift of the angle at which a reflectance minimum occurs can be detected by surface plasmon resonance.

Polished silicon-dioxide-coated silicon wafers or thin silver or gold films on glass substrates are the basis

for the biochemical sensors that are under investigation by Strange and coworkers. Instrument sensitivity enhancement is achieved when green or blue argon ion laser light is used to replace conventionally employed HeNe light, according to Strange.

The instrument has been used to follow antibody-antigen complex formation by monitoring reflectance changes of silicon surfaces coated with human serum albumin before and after incubation with sera containing antihuman serum albumin in varying concentrations. Comparisons were made between wet and dry samples.

According to Strange, high resolution has been achieved with reflectance changes of 0.05 percent being resolved. Read-out is essentially instantaneous and the time required to obtain results is determined only by the incubation times of the reactions under study.

5 BIOCOMPATABILITY

Studies on the interaction between cells and the surface of titanium and Teflon implants were presented by P. Thomsen (Department of Anatomy, University of Göteborg, and the Institute of Physics, Chalmers University of Technology, Göteborg, Sweden). Thomsen stated that in order to understand the biology of the tissue-transplant interface, a detailed knowledge of the mode of interaction between cells and implants is required. Theoretical considerations supported by the experimental observations of Thomsen and coworkers suggested that the early interaction between the implant and inflammatory cells, inevitably present at the site of implant insertion, is of decisive importance for the further changes in the surrounding tissues. Thus, in the present study, Thomsen and his group have examined the ultrastructure of the tissue close to titanium and Teflon implants.

Titanium and Teflon implants were inserted in the abdominal wall of rats. After 8 hours; 1, 3, and 7 days; and 3, 6, and 9 weeks the abdominal wall tissue was fixed by glutaraldehyde perfusion. The implant with surrounding tissue was postfixed in osmium tetroxide and embedded in epoxy resin. The titanium metal (but not the titanium dioxide on the implant surface) was then removed electrochemically. After reembedding, sections were cut on an ultramicrotome. Teflon specimens were cut with the polymer still attached to the embedded tissue.

The layer of titanium dioxide, constituting the surface of the implant, appeared in sections as a dense, 8- to 10-nm wide, continuous line. Six hours after insertion the titanium implants were surrounded by damaged tissue cells and numerous inflammatory cells, predominantly polymorphonuclear granulocytes (PMN). Strands of fiber were attached to the implant surface. PMN were not accumulated at the implant surface. After 1, 3, and 7 days a 10- to 100-um-wide space containing scattered erythrocytes, PMN, and monocytes separated the implant surface from reorganizing connective tissue. Some leukocytes were attached to the implant surface but most of them were not. At later time intervals (3, 6, and 9 weeks) fibroblasts were found in close apposition to the implant surface.

When Teflon specimens were cut, the polymer detached from the tissue; the tissue-implant interface was therefore not as easily and exactly defined as for titanium implants. The acute inflammatory response during the first week was much more pronounced around Teflon implants as compared to titanium implants. The inflammatory cells were not concentrated on the surface of the implant. The pronounced acute response developed into a chronic inflammation with macrophages and multinucleated giant cells--but never fibroblasts--in close apposition to the implant surface.

Thomsen stated that these observations indicate that little direct interaction between inflammatory cells and the surface of titanium and Teflon implants, covered by a proteinaceous material, occurs during the first week after implant insertion. At later times, fibroblasts are in close contact with the implant surface of titanium implants, whereas Teflon implants are still surrounded by inflammatory cells.

Analysis of Inflammatory Exudates in Assessment of Biocompatibility

A report on the analysis of inflammatory exudates in the assessment of biocompatability was presented by D.F. Williams (Institute of Medical and Dental Bioengineering, University of Liverpool, UK). Williams stated that previous studies have shown that the analysis of exudates that accumulate within the vicinity of implanted devices may provide information concerning their biocompatability characteristics. Williams and coworkers have carried out detailed studies in which the inflammatory exudate that forms within the lumen of implanted tubular materials has been analyzed and found to be very informative with respect to subtle differences in biocompatability. Sections of catheters measuring approximately 50 mm in length and internal diameter of 2 to 4 mm were implanted in rats and left for between 1 and 10 weeks. At the end of the test period, the implants were carefully harvested and the exudate forming in the lumen was collected. A blood sample was taken from the tail vein and spun to yield a serum sample. The exudates were examined by assaying marker enzymes, principally alkaline phosphatase, and by protein assay, using the technique of electrofocusing. Specimen studies included a range of elastomeric and rigid catheters including varieties of silicon, polyvinyl chloride, and polyethylene.

The alkaline phosphatase activity within the exudate was reproducibly high at 1 week but decreased exponentially with time as the inflammatory response to the trauma decreased. The rate of loss of activity, however, appeared to be related to the nature of the implanted material, according to Williams, with a logarithmic plot yielding a linear decrease to background level--the rate correlating with the known biocompatability. The isoelectric focusing was carried out with an LKB Multiphor II using Ampholine preformed polyacrylamide gels operating on a pH gradient from 3.5 to 9.5, and the protein profile of the exudates was compared with the serum of the same animal. No new peaks were seen with silicon rubber when the exudate was compared to the serum. However, with silicon, albumin levels were increased and a new peak at pH 6.3 was found. Other catheters of greater irritancy than pure silicon have produced similar differences, according to Williams. Thus, Williams thinks that this method provides quantitative and qualitative methods for assessing small differences in biocompatability.

A Chamber Technique for Study of Cells and Fluids Near Implanted Materials

The development of a chamber technique for quantitative studies of cells and fluid close to implanted materials has been carried out by A.S. Eriksson (Department of Anatomy, University of Göteborg, Sweden). Eriksson stated that nonalloyed titanium is integrated into bone tissue without an intervening layer of connective tissue, and in patients treated for edentulousness an excellent function can be maintained for decades. In order to understand the mechanisms behind the healing-in of biomaterials, experimental studies on the interactions between an implant and surrounding cells are essential. Recent studies by Eriksson and coworkers, using quantitative morphological techniques have provided new information about the healing-in of implants in soft and hard tissues. Their results have suggested that the early interactions between the implant and cells are of great importance for the subsequent structure of the surrounding tissue.

In the present study a chamber technique was developed to enable the examination of small amounts of implant-surrounding cells and extracellular fluids. A porous chamber consisting of a removable distal portion made of pure titanium or Teflon connected to a proximal plate with an attached cylindrical portion, was inserted into the abdominal wall of rats. After a skin excision and removal of a cover screw on top of the cylinder of the titanium chamber, the exudate was retrieved 8 hours to 15 days after surgery (30 rats). The exudate was collected by washing, using an automatic pipette (giving a total of 150 µl). The cells

were counted and their types and proportions determined on cytocentrifuge preparations. After separation of the cells the protein content of the fluid was analyzed electrophoretically. In the titanium chambers the number of leukocytes slowly increased between 8 hours and 9 days. PMN predominated at each time point studied but their relative proportions decreased with time (97 percent at 8 hours, 70 percent at 15 days). Among the mononuclear cells, monocytes were two to four times more frequent than lymphocytes. Teflon chambers were studied 1 to 9 days after insertion (20 rats). The number of inflammatory cells were higher as compared to those of the titanium chambers and already apparent 1 day after insertion. A large increase in the number of cells (mainly PMN) was evident between 3 and 6 days (the amount of cells about four times those present in the titanium chambers). The amount of exudate was higher in the Teflon chambers. Using the electrophoretic techniques it was possible to determine the proteins in the small amount of exudate fluid retrieved from the titanium chambers.

Thus, using the chamber technique described above, it appears that small amounts of cells and fluid close to implanted biomaterials can be analyzed in a quantitative manner. According to Eriksson, this technique is presently being used to increase our knowledge about cellular migration, accumulation, activation, and secretory properties as well as interfacial fluid composition in relation to implanted foreign materials.

6 BIOADHESION

Interaction of Cells with Polymer Surfaces

The interaction of cells with polymer surfaces was discussed by J. Feijen (Department of Chemical Technology, University of Twente, Enschede, the Netherlands). Feijen stated that the interaction of cells with polymer surfaces depends on both the character of the cell and substrate surfaces and on the type of medium used. In order to obtain more insight into the adhesion phenomena, well-characterized substrates are required and the properties of the cell surfaces have to be determined. According to Feijen, adhesion of cells onto polymer surfaces only takes place when the change in the Helmholz free energy is negative. Long-range interactions can be described by the DLVO theory, whereas a proper description of short-range interactions is not yet available. The presence of proteins in the cellular medium usually leads to protein adsorption at the polymer-liquid interface which may strongly influence the adhesion phenomena, according to Feijen.

In order to study cellular interactions with polymer surfaces, Feijen and coworkers synthesized a series of methacrylate (co) polymers with varying surface hydrophobicity, charge, and chain mobility. The surfaces were characterized with the Wilhelmy plate technique (wettability and mobility) and streaming potential measurements (zeta potential). The adhesion of four strains of coagulase-negative staphylococci (CNS) with different surface hydrophobicity and charge onto polymers was studied using a perfusion cell. Feijen and coworkers found that the rate of CNS adhesion onto positively charged surfaces was significantly higher than onto all other surfaces. Introduction of negatively charged methacrylic acid residues into the copolymer did not decrease the rate of CNS adhesion as compared to that on pure poly (methyl methacrylate). Lower rates of adhesion and low plateau values were observed when CNS were exposed to hydrophylic copolymers.

Human endothelial cells (HEC) isolated from umbilical cord were contacted with the same polymers in a modified "Bionique" growth chamber. In the presence of serum containing culture medium, the highest adhesion was found on moderately wettable copolymers. Furthermore, high numbers of HEC were observed on positively charged copolymers. In serum-free medium, HEC adhered onto the charged copolymers but not onto hydrophilic, hydroxyethyl-methacrylate-containing copolymers. Under these conditions, complete

cell spreading was only observed on the positively charged copolymers.

Staphylococcal Adhesion to Cartilage and Collagen in Intra-articular Sepsis

Work on this subject was discussed by A.G. Gristina (Section of Orthopedic Surgery and Department of Pathology, Wake Forest University Medical Center, Winston-Salem, North Carolina). Gristina stated that the ability of certain strains of *Staphylococcus aureus* to specifically bind collagen is believed to be a virulence-related characteristic. Binding to collagen is a high-affinity, strain-specific, reversible interaction. *S. aureus* is an important pathogen in musculoskeletal sepsis, and it has been suggested that binding to collagen is a relevant mechanism in the adherent colonization of cartilage and bone and may be particularly important in the pathogenesis of intra-articular infections and osteomyelitis. Previous studies by Gristina and coworkers have demonstrated the role of microbial adhesion in osteomyelitis and biomaterial-centered infections. In this report Gristina presented scanning electron microscopic (SEM) and transmission electron microscopic (TEM) studies of direct retrievals of tissue from infected joints in man, demonstrating the suggested microbial adhesion to cartilaginous surfaces. In addition, their findings were reproduced in a rabbit model, showing direct bacterial-to-collagen fiber contact. The *in vivo* retrievals and animal studies carried out by Gristina and coworkers suggest that the pathogenesis of joint sepsis is directly related to microbial receptor-collagen adhesion and that bacteria on cartilaginous surfaces are surrounded by extracellular polysaccharides, which form a continuum between the bacteria and the cartilage surface and adjacent aggegating organisms. According to Gristina, his group's studies also suggest that cartilage (and probably collagen) rather than synovium is the specific target for microbial adhesion in joint sepsis. Collagen, as a component of conditioning films about biomaterials may also be a site for microbial adhesion.

Interactions and Bioadhesive Behavior of PAA-Containing Polymer Discs

The interactions and bioadhesive behavior of polymer discs containing polyacrylic-acid (PAA) were studied with bovine sublingual mucus and tissue by G. Ponchel (Laboratoire de Pharmacie Galénique et de Biopharmacie U.A., CNRS 1218, Université de Paris-Sud, Chatenay-Malabry, France). The discs were prepared from PAA and hydroxylpropyl methylcellulose and were surface-preswollen for 10 minutes using water at pH 5.9. The adhesive interaction was measured at 26°C using a modified tensile tester. It was found that the bioadhesive bond strength is a function of the PAA content up to the level of approximately 50 weight percent. The fracture bioadhesion mechanism was used to analyze the results, and the work for adhesion, fracture energy, modulus of the swollen layers, and interfacial fracture depth were measured. Using a diffusional analysis, the penetration depth of PAA chains in the mucus was also calculated and found to be the same as the fracture depth. Thus, during bioadhesion, the rupture of these bonds occurs beyond the mucus/polymer interface. The results were verified by electron microscopic studies. Finally, according to Ponchel, stress-relaxation experiments established the dynamic behavior of the bioadhesive phenomenon.

Interaction of Modified HPMA Copolymers with Rat Intestine

The interaction of modified N-(2-hydroxylpropyl) methacrylate (HPMA) copolymers with adult rat intestine was reported by J.F. Bridges (Department of Biological Sciences, University of Keele, UK). Copolymers of HPMA containing cationic (trimethylammonium chloride) residues or sugars (galactosamine, mannosamine, glucosamine, fucosylamine, or galactose) were synthesized by Bridges and her coworkers and tested *in vitro* as potential intestinal bioadhesives. The cationic ^{125}I-labeled HPMA copolymer was found to exhibit considerable region-independent intestinal bioadhesion. Although incorporation of amino-sugar residues into ^{125}I-labeled HPMA copolymers

produced less tissue association than observed with the cationic derivative, these sugar-containing polymers showed binding. Incorporation of galactosamine residues potentiated bioadhesion in the duodenum and the first part of the jejunum, whereas fucosylamine potentiated bioadhesion in the third part of the jejunum. Bioadhesion was increased if the amino-sugar was bound to the polymer backbone via a nondegradable glycylglycine spacer, rather than directly conjugated to the polymer backbone. Studies with copolymer-conjugated colloid gold probes showed that the copolymers markedly adhere to the acid mucopolysaccharide portion of the intestinal mucin.

Bridges stated that preliminary *in vivo* experiments have supported *in vitro* data. Following oral administration of ^{125}I-labeled HPMA copolymers to rats (1 hour), unmodified polymer showed 60 percent of the dose administered in the small intestine (all in the ileum). Incorporation of galactosamine residue slowed transit, 72 percent of the dose present in the small intestine after 1 hour, was equally distributed in the stomach, duodenum, and jejunum but not detected in the ileum. HPMA copolymers containing fucosoylamine had the slowest transit time; 97 percent of the dose was still present in the small intestine after 1 hour, and of this 73 percent was located in the jejunum.

Adhesion of Endothelial Cell to Vascular Prosthetic Surfaces

Studies of endothelial cell adhesion to vascular prosthetic surfaces were presented by D. Gourevitch (Department of Surgery and Pathology, East Birmingham Hospital, University of Birmingham, UK). Improving the long-term patency of small diameter prosthetic grafts (less than 4 mm, internal diameter) remains an important but elusive objective in cardiovascular surgery, according to Gourevitch. In pursuit of a nonthrombogenic surface, Gourevitch and coworkers have cultured human adult endothelial cells (HEC), and examined their adhesive properties and their ability to colonize the inner surface of a Dacron graft.

HEC were harvested and cultured on plastic petri dishes. To examine cell adhesion, endothelial cells were labeled with Indium and innoculated onto Dacron wells which had been prepared with either cold insoluble globulin (CIG), 1 percent gelatin, alginate, or left untreated as control. At 20-minute intervals, the wells were irrigated and the radioactivity of the supernatant was measured. The mean percentage cell adhesion to CIG and gelatin at 100 minutes was 83.2 and 82 percent respectively, whereas alginate at 61.8 percent and the control at 53.5 percent showed less adhesion.

In similarly prepared Dacron wells, growth was allowed to continue for 96 hours at 37°C. Cells were released with trypsin EDTA and counted using a hemocytometer in conjunction with the trypan blue exclusion test. Cell growth exceeded 2.5×10^5 cells/ml on CIG and gelatin, but was never more than 5×10^4 cells/ml when using alginate or an uncoated Dacron surface.

Finally, endothelial cells were subcultured at a high concentration (greater than 3.3×10^5 cells/ml) onto CIG pretreated Dacron prosthetic material, as square pieces (1×1 cm) and tubes (3 cm×5 mm internal diameter). Scanning electron microscopy showed cell growth on all 17 Dacron squares and on seven of the 12 tubes. The endothelial cell monolayer was confluent in 10 of the Dacron squares and in five tubes. Thus, the results showed that cultured endothelial cells adhere most effectively to Dacron vascular grafts pretreated with CIG, and can develop as a confluent monolayer to form a natural flow surface.

Adhesion of Cells to Silicon Substrate

Studies on cell adhesion to silicon substrate were reported by M. Grattarola (Biophysical and Electron Engineering Department, SGS Microelectronics, Genoa, Italy). According to Grattarola, the controlled growth of cellular assemblies on integrated circuits is a challenging goal, with far-reaching implications in both biology and electronics. He thinks that one of the primary steps to be taken towards this goal is the characterization

of the adhesion of cells to silicon substrata.

Grattarola and his group studied the adhesion of human red blood cells (rbc's) to silicon derivatives. They also started to grow, on silicon derivatives, cells of the rat PC12 line. In the case of rbc's, cell adhesion to silicon wafers (covered with dioxide of various depths and with various dopins) was compared with adhesion to glass. The adhesion of the rbc's was tested by allowing the cells to settle for 50 minutes onto the substrata, which were inserted in a revolving bar. The bar was then revolved and the rbc's were allowed to detach under gravity for a 30-minute period. The adhering cells were viewed before and after the treatment under reflection microscopy conditions by means of a television camera. The digitalized images were then analyzed and the percentage of adhering cells evaluated. Buffers of two different ionic strengths were used.

Under these experimental conditions, the adhesion of rbc's to silicon dioxide was found to compare favorably with that to glass substrata. The cell behavior at different ionic strengths suggested the existence of molecular contacts between cells and substrata, according to Grattarola. This is a crucial prerequisite for a functional coupling of living cells to integrated circuits. In the case of PC12 cells, their growth on silicon substrata was compared to the growth under standard conditions. Grattarola stated that the preliminary data obtained seem to indicate a comparable growth.

7 DRUG DELIVERY SYSTEMS

Polymeric Microspheres as Drug Carriers

A review of polymeric microspheres as drug carriers was presented by S.S. Davis (Department of Pharmacy, University of Nottingham, UK). Davis stated that in research on drug delivery the following requirements must be met for an effective drug delivery method. These are that the drug systems must: (1) accumulate at the required site; (2) be released at the appropriate site; (3) must be stable *in vitro* (adequate shelf-life); and (4) be nontoxic and biodegradable and so must be nonimmunogenic. According to Davis, solution of these requirements is a difficult problem. The levels of targeting include the following: (1) organ, (2) cell, and (3) intracellular (nucleus, Golgi, cytoplasm, lysosomes). In the liver, for example, one may want to target a drug to a discrete part of the organ such as the Kupfer or endothelial, or parenchymal cells.

Although, for example, a great advantage of using polymeric microspheres as drug carriers is that the drug could be delivered directly to tumor cells, there are many problems to be resolved. Davis cited some examples such as: (1) the anatomical location of the tumor, (2) blood supply to the tumor, (3) the structure and permeability of the tumor microcirculation, and (4) the carrier system and sequestration (by other sites in the body such as the reticuloendothelial system). Some barriers to drug targeting include capture by the reticuloendothelial system and escape from the vascular system. Foreign material (drug delivery system) may also be captured by monocytes and circulating macrophages in the blood.

Studies on the use of adsorbed and grafted copolymers have shown that copolymers can alter processes of uptake onto particle surfaces (opsonization) as well as the interaction of particles with macrophages (cell-particle adhesion). It has been found that the selection of the appropriate coating material makes it possible to direct particles within the body to organs such as the lung, liver, bone marrow, or to retain materials within the systemic circulation.

Biodegradable Hollow Fibers for Controlled Release of Drugs

A report on biodegradable hollow fibers for the controlled release of drugs was presented by J.M. Schakenraad (Laboratory for Histology and Center for Medical Electron Microscopy, University of Groningen, the Netherlands). The research was carried out as a collaborative project with scientists from the University of Twente, Enchede, and the Plastics and

Rubber Research Institute, Delft, the Netherlands. These investigators recently developed hollow fibers of poly-L-lactic acid (external diameter 0.7 mm, wall thickness of 0.05 mm) for controlled release of highly active drugs over periods of more than 9 months. The fibers are injected subcutaneously and can easily be removed, if necessary, by a small incision, according to Schakenraad. The prime feature of this reservoir device is its zero order drug release; using a dry-wet coagulation spinning process, the membrane permeability can be adjusted to achieve optimal release rates for a wide variety of drugs such as pregnancy control drugs, cytostatics, leutinizing releasing hormone, etc. After release of the drug, the poly-L-lactic acid fiber should degrade completely, *in situ*, according to Schakenraad.

In their pilot model, Schakenraad and coworkers filled the fibers with a pregnancy control drug (levonorgestrel) suspended in castor oil, and both ends were heat-sealed. *In vivo* release studies in rats showed a zero order release of 2.4 µg/day/fiber. Biodegradation of the fibers was measured by determination of molecular weight and by stress-strain experiments. A 65-percent loss of mechanical strength within 4 months was observed without functional loss. No degradation was observed morphologically up to 6 months.

According to Schakenraad, tissue reaction towards the polylactic fiber can be described as a very moderate foreign body reponse. These cells were very active as judged by the presence of mitochondria, Golgi apparatus, endoplasmic reticulum, and many vesicles. At 2 months after implantation, a cell-poor area mainly containing collagen and few fibroblasts was observed around the fiber. In all stages after implantation, blood vessels were observed growing towards the fiber, ensuring the uptake of hormone in the blood.

A New Bioerodible Polyphosphazene Matrix System for Controlled Release

Studies on controlled release using a new bioerodible polyphosphazene matrix system were presented by C.T. Laurencin (Department of Applied Biological Sciences, Massachusetts Institute of Technology). This research was carried out in collaboration with scientists from the Department of Chemistry, Harvard University, and Pennsylvania State University. Polyphosphazenes are high-molecular-weight polymers with backbones consisting of nitrogen and phosphorous atoms separated by formally alternating single and double bonds. According to Laurencin, their potential for biomedical applications stems from the fact that polymers with a wide array of properties can be synthesized using the same starting compound, poly(dichlorophosphazene), through changes in side chain substituents. Many of these polymeric materials that have been synthesized have been found to biodegrade to harmless products.

Laurencin and coworkers designed a novel monolithic bioerodible polyphosphazene matrix system, and they carried out preliminary studies assessing its potential for use in drug delivery. The system utilized poly (imidazole methylphenoxy) phosphazene in formulations consisting of 20 percent and 45 percent substituted imidazole side chain groups respectively. Both polymers showed reproducible degration profiles that were dependent on imidazole content. Using p-nitoaniline as a model molecule, release from polyphosphazene matrices appeared to follow diffusional release kinetics. A study of *in vitro* and *in vivo* release took place for approximately 1000 hours with the *in vivo* profile demonstrating a lag period of approximately 80 hours. Polypeptide release was demonstrated using bovine serum albumin. Release from these matrices was characterized by a burst of almost 25 percent followed by a steady release for over 1000 hours. This burst of release was eliminated through the inclusion of a coating step in fabrication.

Electron microscopy studies of 20-percent imidazole-substituted polymers revealed their surfaces to be smooth with only minor irregularities. After 1000 hours of degradation, matrices took on a more irregular appearance; however, no

large crevices or defects were noted. Preliminary histological studies of the 20-percent imidazole-substituted polymers subcutaneously implanted in rats for 1 month revealed intact polymer surrounded by a fibrous capsule. No gross areas of surrounding inflammation were found.

Laurencin thinks that through the use of this novel monolithic polyphosphazene matrix system, low-molecular-weight molecules as well as polypeptides can be reproducibly released over prolonged periods of time. He stated that the versatility found in this system presents possibilities for a variety of controlled release applications.

Imaging of Biomaterials and Drug Delivery Systems

A discussion of methods for the imaging of biomaterials and drug delivery systems was given by M.C. Davies (Department of Pharmacy, University of Nottingham, UK). According to Davies, surface reaction and interactions are critical phenomena in the in-life performance of many biomaterials. The study of interfacial chemistry requires the availability of suitable techniques for the characterization of such surfaces. Davies presented an overview of the potential of static secondary ion mass spectrometry (SSIMS) and SIMS imaging for the surface chemical analysis of polymeric surfaces employed in biomaterials and drug delivery technologies. SSIMS provides a mass spectrum of the surface which may be readily interpreted by normal mass spectrometry rules. According to Davies, the high sensitivity and degree of molecular specificity of the technique can provide a unique insight on the surface chemical structure of polymeric biomaterial surfaces.

SSIMS permits the qualitative and quantitative characterization of the surface chemical morphology of polymeric biomaterial interfaces of natural (for example, hydroxypropyl cellulose) and synthetic (for example, polylactide) origin, and their subsequent modification by chemical treatments such as grafting, oxidation, and glow discharge treatments to improve biocompatability. Similarly, SSIMS can permit the qualitative and semiqualitative determination of the surface presence of molecules--for example, drugs, plasticizers, contaminants, etc. SSIMS may also be employed to gain an insight into the nature of protein deposition on polymeric surfaces; i.e., even or patchwise distribution of the protein molecules.

SIMS imaging provides two-dimensional chemical mapping of the surface of a biomaterial, permitting the elucidation of the ditribution of molecules--for example, drugs, mould lubricants, etc.--and protein deposition on a biomaterial surface.

A report on the use of a soluble synthetic polymer for targeting and controlled release of anticancer agents was presented by R. Duncan (Department of Biological Sciences, University of Keele, UK). This was a collaborative study with researchers at the Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, Prague, Czechoslovakia. Conjugates of N-(2-hydroxylpropyl)methacrylate)HPMA) copolymers and anticancer agents daunomycin (DNM), adrymicin (ADR), and sarcolysin (SLE, also called mephalan) were synthesized and tested against a variety of *in vitro* tumors (L1210, CRF, hepatoma, melanoma and Walker sarcoma). Activity against tumor cells *in vitro* was shown to be dependent on the peptidyl side chain used to bind polymer to drug as well as on the drug used (certain cells were more sensitive to one particular compound) and on the incorporation of molecules into the drug-conjugate (carbohydrates, hormones, or proteins) shown to promote specific cell-surface binding, i.e., targeting.

HPMA copolymer-DNM or -ADR conjugates administered intraperitoneally were found to be very affective against 10^5 L1210 cells administered intraperitoneally to DBA mice if the drug was bound via a biodegradable peptide spacer. Nondegradable conjugates were completely inactive. Incorporation of carbohydrate residues (galactosamine or fucosylamine) potentiated tumor activity under certain experimental conditions. It was also found that conjugated drugs could be

administered at more than 10 times the optimum tolerated dose of free drug with no apparent toxicity. Drug conjugates administered intraperitoneally were also shown to increase the life span of DBA mice bearing a L1210 tumor subcutaneously. Thus, preparation of radiolabeled polymer conjugates has enabled a comparative study of the pharmacokinetics of free and polymer-bound drug. Furthermore, both the British and Czech groups have patent applications for their synthetic polymeric anticancer drugs.

8 CONCLUSIONS

This interesting and timely conference on the study of interactions between biotechnology and materials showed that fundamental studies of cell surface/activity have their application in clinical environments and that new molecules introduced through biotechnology can influence cell activity. Furthermore, it was apparent that new techniques for observing and measuring such behavior are constantly being developed.